# Exhibit 6

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# Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential

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## Contents



## Summary

Nitrosamines form a large group of genotoxic chemical carcinogens which occur in the human diet and other environmental media, and can be formed endogenously in the human body. *N*-Nitroso compounds can induce cancer in experimental animals. Some representative compounds of this class induce cancer in at least 40 different animal species including higher primates. Tumours induced in experimental animals resemble their human counterparts with respect to both morphological and biochemical properties. Extensive experimental, and some epidemiological data suggest that humans are susceptible to carcinogenesis by *N*-nitroso compounds and that the presence of these compounds in some foods may be regarded as an aetiological risk factor for certain human cancers including cancers of the oesophagus, stomach and nasopharynx.

## Introduction

It is generally accepted that cancer incidences in various organs show strong geographical varia-

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TABLE 1

*N*-NITROSO COMPOUNDS IN THE DIET, CARCINOGENICITY AND OCCURRENCE

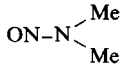
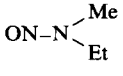
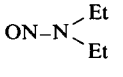
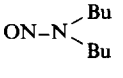
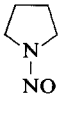
| *N*-Nitroso compound | Carcinogenicity (following oral administration) | | Major dietary source (μg/kg) | | |
|---|---|---|---|---|---|
| | Species | Organotropy | Foodstuff | Concentration | |
| | | | | Range | Mean |
| *N*-Nitrosodimethylamine (NDMA) | Rat | Liver, kidney, (lung) | Cured meats | 1.0–5.0 | |
| | Mouse | Liver, kidney, lung | Fried bacon | < 23 | 4.0 |
| | Hamster | Liver, (glandular stomach) | Marine products: | | |
|  | Guinea pig | Liver | Dried fish (Japan) | 3.0–39 | 8.6 |
| | Rabbit | Liver, lung | Dried fish (Greenland) | 8.6–38 | |
| | Mink | Liver | Dried shrimps (China) | 5.4–132 | 43.9 |
| | | | Broiled squid (China) | < 300 | |
| | | | Millet flour and grain products (China) | 0.1–1.3 | 0.5 |
| | | | Dairy and cheese products | 1–6 | |
| | | | Dried milk products | < 7.0 | |
| | | | Edible oils and fats | < 1.0 | |
| | | | Pickled/fermented vegetables | < 5.0 | |
| | | | Beer | < 0.5 | 0.2 |
| | | | Alcoholic beverages (whisky) | < 2.0 | 1.0 |
| *N*-Nitrosoethylmethylamine (NEMA) | Rat | Liver, nasal cavity, (oesophagus) | Pickled/fermented vegetables (China) | < 5.0 | |
| | Hamster | Liver, nasal cavity, trachea | | | |
|  | | | | | |
| *N*-Nitrosodiethylamine (NDEA) | Rat | Liver, kidney, (oesophagus) | Cured meats (packed in rubber nettings) | < 2.4 | |
| | Mouse | Liver, lung, oesophagus, forestomach | Salami | | |
|  | Hamster | Trachea, lung, nasal cavity, oesophagus, forestomach, liver | | < 3.9 | 0.6 |
| | | | Millet flour and grain products (China) | 0.1–0.5 | 0.22 |
| | Rabbit, cat | Liver, oesophagus | | | |
| | Guinea pig, dog, monkey | Liver | Dried cuttlefish | < 4.5 | 1.4 |
| *N*-Nitrosodibutylamine (NDBA) | Rat | Liver, urinary bladder, (oesophagus, pharynx) | Cured meats (packed in rubber nettings) | 1–56 | 10.2 |
| | Mouse | Forestomach, liver, oesophagus, urinary bladder, (lung) | Smoked chicken | < 5.3 | |
|  | | | Dried fish (Japan) | < 3.1 | |
| | Hamster | Respiratory tract, lung, urinary bladder | | | |
| | Guinea pig | Liver, urinary bladder | | | |
| *N*-Nitrosopyrrolidine (NPYR) | Rat | Liver, nasal cavity, (vagina, testis) | Cured meats | 1.0–5.0 | 1.8 |
| | Mouse | Lung | Fried bacon | < 130 | 17 |
|  | | | Pickled vegetables | < 96 | |
| | | | Mixed spices | < 10 | |
| | | | Dried chillies | < 6.0 | 3.1 |
| | | | Broiled squid | 2.4–13 | |

TABLE 1 (continued)

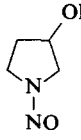
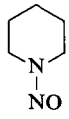
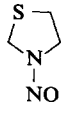
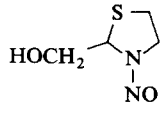
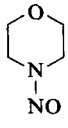
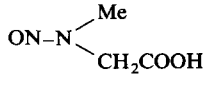
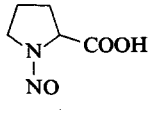
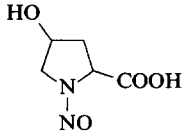
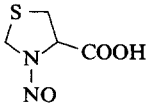
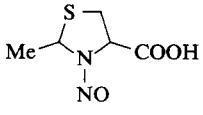
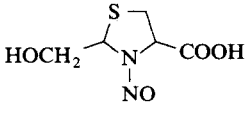
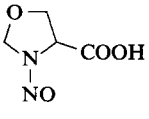
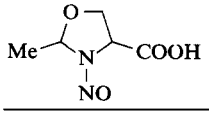
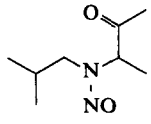
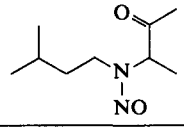
| *N*-Nitroso compound | Carcinogenicity (following oral administration) | | Major dietary source (μg/kg) | | |
|---|---|---|---|---|---|
| | Species | Organotropy | Foodstuff | Concentration | |
| | | | | Range | Mean |
| *N*-Nitroso-3-hydroxy-pyrrolidine (NHPYR)  | Rat | Liver | Cured meats<br>Fried bacon | < 7.0<br>0.4–3.9 | <br>2.2 |
| *N*-Nitrosopiperidine (NPIP)  | Rat | Liver, oesophagus, upper respiratory and digestive tracts, nasal cavity | Cured meats<br>Fried bacon<br>Peppered salami | < 20<br>< 9.2<br>< 30 | <br><br>5.8 |
| | Mouse | Forestomach, liver, lung | Pepper | < 300 | |
| | Hamster | Liver, upper respiratory and digestive tracts | Mixed spices<br>Pickled vegetables | 0.6–3.5<br>< 14 | |
| *N*-Nitrosothiazolidine (NTHZ)  | Rat | Non-carcinogenic | Cured meats<br>Fried bacon<br>Smoked fish<br>Smoked oyster | < 32<br>< 30<br>< 6<br>< 109 | <br>8.9 |
| *N*-Nitroso-2-hydroxymethyl-thiazolidine (NHMTHZ)  | | No data available | Cured meats<br>Smoked ham | Occasionally<br>< 2.8 | |
| *N*-Nitrosomorpholine (NMOR)  | Rat<br>Mouse<br>Hamster | Liver, (kidney, ovary)<br>Liver, lung<br>Liver, upper respiratory tract, colon | Packaging contamination of:<br>Fats/margarine<br>Dairy products | <br>1.7–3.8<br>< 3.2 | |
| *N*-Nitrososarcosine (NSAR)  | Rat<br>Mouse | Oesophagus<br>Lung, nasal cavity, (small intestine) | Cured meats<br>Pickled vegetables<br>Brewing malt | < 410<br>< 36<br>Occasionally | |
| *N*-Nitrosoproline (NPRO)  | Rat<br>Mouse | Non-carcinogenic<br>Non-carcinogenic | Cured meats<br>Preserved fish<br>Broiled squid<br>Dried vegetables<br>Dried chillies<br>Brewing malt<br>Beer | 20–580<br>< 89<br>< 94<br>< 24<br>< 132<br>< 113<br>1–6 | 140<br><br><br><br><br><br>1.7 |

TABLE 1 (continued)

| *N*-Nitroso compound | Carcinogenicity (following oral administration) | | Major dietary source (μg/kg) | | |
|---|---|---|---|---|---|
| | Species | Organotropy | Foodstuff | Concentration | |
| | | | | Range | Mean |
| *N*-Nitroso-4-hydroxy-proline (NHPRO)  | Rat<br>Mouse | Non-carcinogenic<br>Non-carcinogenic | Cured meats | 10–560 | |
| *N*-Nitrosothiazolidine-4-carboxylic acid (NTCA)  | | No data available | Cured meats<br>Fried bacon<br>Smoked poultry<br>Smoked fish<br>Smoked oyster<br>Smoked cheese | <1620<br><14,000<br><1240<br><1600<br><167<br>5–24 | <br>85<br><br>67<br><br> |
| *N*-Nitroso-2-methylthiazolidine-4-carboxylic acid (NMTCA)  | | No data available | Cured meats<br>Smoked poultry | < 28<br>< 98 | <br> |
| *N*-Nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic acid (NHMTCA)  | | No data available | Cured meats<br>Smoked cheese<br>Smoked poultry | < 2100<br><1628<br>< 462 | <br> |
| *N*-Nitrosooxazolidine-4-carboxylic acid (NOCA)  | | No data available | Cured meats | 40–70 | |
| *N*-Nitroso-5-methyloxazolidine-4-carboxylic acid (NMOCA)  | | No data available | Cured meats | 30–120 | |

tions and that environmental factors, including the human diet, may be an important cause of human cancer (Wynder and Gori, 1977; Doll and Peto, 1981). Current knowledge on the exposure to environmental *N*-nitroso compounds has seen considerable advancement since Druckrey and Preussmann (1962) first postulated that the formation of carcinogenic nitrosamines could occur in

TABLE 1 (continued)

| *N*-Nitroso compound | Carcinogenicity (following oral administration) | | Major dietary source (μg/kg) | | |
|---|---|---|---|---|---|
| | Species | Organotropy | Foodstuff | Concentration | |
| | | | | Range | Mean |
| *N*-Nitroso-*N*-(1-methylacetonyl)-2-methylpropylamine (NMAMPA)  | Mice | Forestomach (tested by feeding with amine + nitrite) | Moldy millet and wheat flour | <1.2 | |
| *N*-Nitroso-*N*-(1-methylacetonyl)-3-methylbutylamine (NMAMBA)  | | No data available | Millet flour and grain products (China) | 0.1–1.3 | 0.2 |

tobacco smoke via the interaction of nitrogen oxides and tobacco amines. The first validated confirmation of the environmental occurrence of *N*-nitrosamines was provided by Ender et al. (1964) who found traces of *N*-nitrosodimethylamine (NDMA) in nitrite-preserved herring used in sheep fodder following an outbreak of toxic hepatosis in sheep. Concern over the possible health risks posed by these compounds was reinforced following the work of Sander (1967) who provided the first unequivocal proof that endogenous formation of nitrosamines (i.e., their in vivo synthesis from precursor compounds) occurred in experimental animals.

Following the development of a highly sensitive nitrosamine-selective detector (thermal energy analyser, TEA) by Fine et al. (1975), it was soon established that human exposure to carcinogenic *N*-nitroso compounds can occur through the diet. The occurrence of *N*-nitroso compounds in foodstuffs probably represents the most comprehensively researched exposure situation for any class of genotoxic carcinogenic compounds in the human diet, and has been a subject of several recent reviews (Forman, 1987; Hotchkiss, 1987, 1989; Tricker and Preussmann, 1988). The carcinogenic effects of these compounds in experimental animals (Preussmann and Stewart, 1984; Lijinsky, 1987) and their relevance to human cancer (Bartsch and Montesano, 1984; Magee, 1989) have also been extensively reviewed.

## Nitrosamines in the diet

### *Formation of N-nitroso compounds in foodstuffs*

The chemistry of nitrosation of amines in foods is a complex process and has been the subject of many reviews (Challis and Challis, 1982; Williams, 1983; Tricker and Kubacki, 1990). Therefore, the subject will not be reviewed in depth. It is sufficient to mention that oxides of nitrogen (in oxidation states +3 and +4) which can be formed during food processing, preservation and preparation can react with amino compounds and other nucleophiles to produce *N*-, *C*-, *O*-, and *S*-nitroso compounds. The two major sources of oxides of nitrogen (nitrosating agents) result from (I) the

addition of nitrate and/or nitrite to foods, and (II) the heating and/or drying of foods in combustion gases in which molecular nitrogen can be oxidised to oxides of nitrogen.

Nitrosation reactions can be influenced by the presence of nitrosation inhibitors (redox compounds such as ascorbate and vitamin E) and catalysts (metal ions, carbonyl compounds and nucleophilic anions such as $Cl^-$, $I^-$ and $SCN^-$). In plant-based foods, phenolic compounds can catalyse and inhibit nitrosation depending on their structure.

*Occurrence of N-nitroso compounds in the diet*

Over the last decade, more than 500 publications on the occurrence of *N*-nitroso compounds in foodstuffs have been published and several in-depth reviews have recently been compiled (Forman, 1987; Hotchkiss, 1987, 1989; Tricker and Preussmann, 1988). The chemical stability of volatile nitrosamines, and the simplicity of their determination by distillation techniques followed by gas chromatography, has resulted in the analysis of this group of *N*-nitroso compounds in almost all Western foods. In a comprehensive survey of the West German market, Spiegelhalder et al. (1980) analysed 2826 food samples; only 3 volatile nitrosamines, namely *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosopiperidine (NPIP) were regularly found, all other volatile nitrosamines were present only in rare cases. NPYR and NPIP at concentrations above 0.5 μg/kg (ppb) were found in only 3% and 2% of all foods, respectively. However, NDMA was detected in 30% of all samples, 6% of which contained concentrations above 5 μg/kg. Currently available data suggest that Asian foods are more frequently found to contain volatile nitrosamines and at concentrations higher than those found in Western foods. The occurrence of *N*-nitroso compounds in foods, representative levels for individual *N*-nitroso compounds, and the corresponding carcinogenicity data (following oral administration in experimental animals) are summarised in Table 1.

Critical analysis of the data in Table 1 shows that the foodstuffs most commonly contaminated with *N*-nitroso compounds can be classified into several broad groups:

- Foodstuffs preserved by addition of either nitrate and/or nitrite, namely cured meat products (in particular bacon) and cheeses. This is as expected since both methods of preservation introduce nitrosating species into the food matrix.
- Foodstuffs preserved by smoking such as fish and meat products. In this case, oxides of nitrogen present in smoke act as nitrosating agents.
- Foodstuffs subject to drying by combustion gases (containing oxides of nitrogen) such as malt for the production of beer and whiskey, low-fat dried milk products and spices.
- Pickled and salt-preserved foods, in particular plant-based products (pickled vegetables) in which microbial reduction of nitrate to nitrite occurs.
- Foodstuffs stored under humid conditions favouring fungal contamination, particularly the growth of *Fusarium moniliforme*.
- Migration and formation of nitrosamines from food contact materials.

The last source of *N*-nitroso compounds in foods has been a major health concern for infants using rubber pacifiers and/or baby feeding bottles fitted with rubber nipples. Nitrosamines present in rubber formulations can migrate into baby foods and drinks (Spiegelhalder and Preussmann, 1982) and into meat products packed in rubber nettings (Sen, 1988).

Analytical methods for the detection of non-volatile *N*-nitroso compounds in foods are limited to the detection of simple *N*-nitrosated amino acids and their derivatives (Sen and Kubacki, 1987). The occurrence of non-volatile nitrosamines in foods has recently been reviewed (Tricker and Kubacki, 1990) from which the following summary is derived. A wide range of *N*-nitrosated amino acids and their derivatives (see Table 1) are commonly found in cured meats (Tricker et al., 1984a). The presence of several other non-volatile *N*-nitroso compounds is probable in foods and beverages. Examples of such compounds include *N*-nitrosated dipeptides or polypeptides with N-terminal proline (Kubacka et al., 1984; Tricker et al., 1984b) or hydroxyproline residues, *N*-nitrosated 3-substituted indoles (Ahmad et al., 1985), *N*-nitrosated derivatives of certain pesticides (e.g.,

*N*-nitrosocarbaryl, *N*-nitrosoatrazine and *N*-nitrosoglyphosate), non-volatile hydroxylated nitrosamines formed by the nitrosation of spermine (Hotchkiss et al., 1977) and similar compounds, *N*-nitrosated glycosylamines and Amadori compounds (Röper et al., 1982).

*Exposure to N-nitroso compounds*

The dietary exposure to *N*-nitrosodimethylamine (NDMA) and other volatile *N*-nitrosamines has been calculated in a number of food surveys (Table 2). However, it should be taken into consideration that exposure estimates of this kind suffer from uncertainties in food consumption trends averaged over a population. Reductions in the use of nitrates and nitrites used for curing meats and modification of malting techniques in the brewing industry have resulted in significant reductions in the levels of *N*-nitroso compounds over the last 5 years. In West Germany, the daily NDMA exposure from beer of 0.74 μg/day in 1979/1980 (Spiegelhalder et al., 1980) has been reduced to 0.1 μg/day in 1987 (Frommberger, 1989). Thus the current NDMA exposure levels are probably lower than the values quoted in Table 2.

For most Western countries, the exposure to volatile nitrosamines from the diet is generally in the order of 0.3–1.0 μg/day. In developing countries, particularly China, the occurrence and concentrations of volatile nitrosamines in common dietary items (Singer et al., 1986; Lu et al., 1986; Poirier et al., 1987; Song and Hu, 1988) are considerably higher than in Western foods and an increased daily exposure to volatile nitrosamines would be expected.

The daily exposure to currently identified non-volatile *N*-nitrosamines in the diet cannot be directly evaluated, however an exposure estimate of 10–100 μg/day would not seem unreasonable. The majority of these compounds are biologically inactive and are excreted via the urine (reviewed by Bartsch et al., 1989).

## Carcinogenicity of *N*-nitroso compounds

Over 300 *N*-nitroso compounds have been shown to be carcinogenic in one or more animal species (Preussmann and Stewart, 1984; Lijinsky, 1987) and more than 40 animal species including higher primates are susceptible to *N*-nitroso compound-induced carcinogenesis (Bogovski and Bogovski, 1981). Druckrey et al. (1967) observed that tumours induced by *N*-nitroso compounds in experimental animals showed similar morphological properties to tumours found in the correspond-

TABLE 2

DIETARY SURVEYS AND DAILY NDMA INTAKE

| Country | NDMA intake (μg/day) | Major NDMA source | Reference |
|---|---|---|---|
| U.K. | 0.53 [a] | Cured meats (81%) | Gough et al. (1978) |
| The Netherlands | 0.38 | Beer (71%) | Stephany and Schuller (1980) |
| F.R.G. (1979/1980) | 1.02 (men)<br>0.57 (women) | Beer (65%)<br>Cured meats (10%) | Spiegelhalder et al. (1980) |
| F.R.G. (1981) | 0.53 (men)<br>0.35 (women) | Beer (40%)<br>Cured meats (18%) | Spiegelhalder (1983) |
| Japan | 1.8 | Dried fish (91%) | Maki et al. (1980) |
| Japan | 0.5 | Fish products (88%) | Yamamoto et al. (1984) |
| Sweden | 0.29 | Meat products (61%)<br>Beer (30%) | Österdahl (1988) |
| Finland | 0.08 [b] | Smoked fish (75%) | Penttila et al. (1990) |
| China | No data | Marine foods | Song and Hu (1988) |
| Italy | No data | Cured meats | Gavinelli et al. (1988) |

[a] Beer not included in the survey.
[b] Based on limited data.

ing human organs. Recent investigations show that *N*-nitrosobis-(2-oxopropyl)amine-induced pancreatic tumours in the Syrian golden hamster show not only morphological similarities to human pancreatic tumours, but also exhibit similar biochemical properties as well as expression of some tumour-associated antigens with blood group specificities (Pour et al., 1986).

The carcinogenic potential of *N*-nitroso compounds found in foods, following oral administration to experimental animals, has been summarised in Table 1. The organotropism of *N*-nitroso-*N*-(1-methylacetonyl)-2-methylpropylamine can only be assumed from feeding studies of the corresponding amine and nitrite to mice.

## Mechanisms of carcinogenesis

*N*-Nitrosamines per se are stable under physiological conditions and require metabolic activation by cytochrome P450-dependent hydroxylation at the carbon adjacent to the *N*-nitroso group to yield an α-hydroxynitrosamine. Spontaneous elimination of an aldehyde by cleavage of the carbon–nitrogen bond produces an alkyldiazohydroxide as shown in Fig. 1. *N*-Nitrosamides and their related compounds are chemically active under physiological pH conditions and decompose to form the same alkyldiazohydroxide species as an intermediate step in the production of an electrophilic alkyl diazonium ion, the ultimate carcinogen, which can react at nucleophilic sites of various cellular constituents. The alkylation of DNA is generally considered to be the critical cellular target for carcinogens in the initiation of cancer.

Alkylation of DNA by dialkylnitrosamines results in a range of modified bases. In the rat liver, O'Connor et al. (1979) have identified 90% of the methylated products produced by the metabolic activation of NMDA as 3-, 7-, and $O^6$-alkylguanine (0.9%, 66.8%, 6.1%); 1-, 3-, and 7-alkyladenine (0.9%, 2.3%, 0.7%); 3-alkylcytosine (0.6%); $O^4$-alkylthymine (trace) and alkylphosphate triesters (12%). The relative proportions of alkylation at N and O atoms of both purine and pyrimidine bases depend on the alkylating agent and the specificity of the P450 activation in different tissues. The results of several in vitro studies suggest that *N*-nitroso compounds exhibit similar biological activity in human and animal tissues. Montesano and Magee (1974) have shown that liver slices from various species including humans can metabolise NDMA (Table 3). More recent studies have shown that explanted cultures of hu-

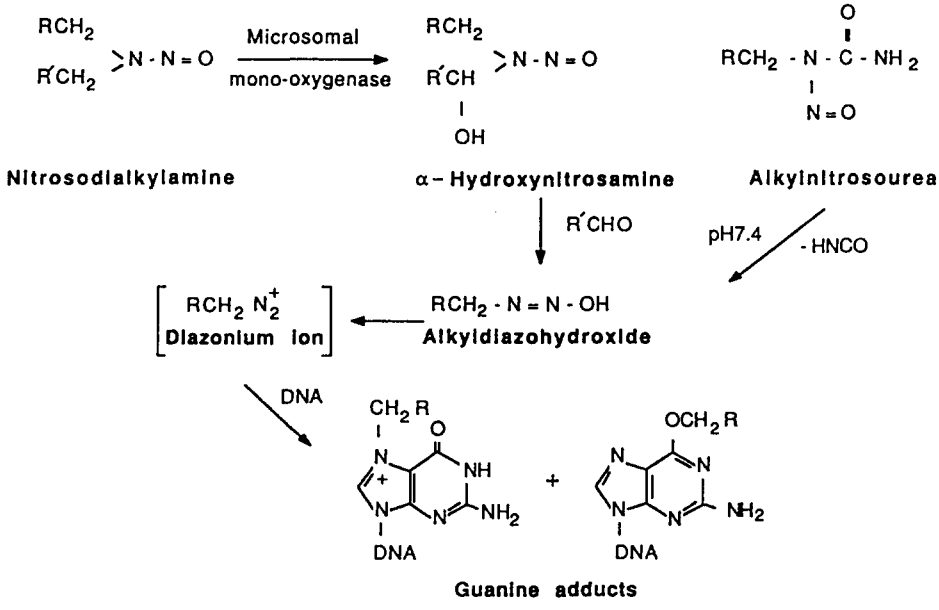



Fig. 1. Metabolic activation of *N*-nitroso compounds.

TABLE 3

COMPARATIVE IN VITRO METABOLISM OF NDMA IN THE LIVER OF DIFFERENT SPECIES

| Species | Relative activity [a] |
|---|---|
| Syrian golden hamster | 100.0 |
| Rat | 65.0 |
| Human | 45.0 |
| Monkey | 6.1 |
| Trout | 0.1 |

[a] Expressed as the percentage formation of 7-methylguanine and [$^{14}$C]carbon dioxide observed in hamsters.

Original data from Montesano and Magee (1974).

man tissues (bronchus, oesophagus, urinary bladder, colon and pancreas) are all able to metabolise simple symmetrical dialkylnitrosamines such as NDMA and NDEA, but not all tissues are able to metabolise cyclic and asymmetrical dialkylnitrosamines. Furthermore, large quantitative differences in metabolic rates (up to 150-fold) occur between individuals (reviewed by Harris et al., 1982).

Not all base modifications are biologically important; the alkylation of the 7-position of guanine, the most abundant modified base in DNA produced by dialkylnitrosamines, shows no correlation with carcinogenic activity. A significant proportion of alkylated DNA products are repaired by either hydrolysis or enzymic apurination or apyrimidation. The formation and persistence of $O^6$-alkylguanine (Pegg, 1983) and $O^4$-alkylthymine (Dyroff et al., 1986) residues are considered to be important base modifications which result in the incorporation of non-complementary bases (mis-coding) during polyribonucleotide or polydeoxyribonucleotide synthesis. The significance of $O^6$-alkylguanine has been clearly demonstrated in carcinogenesis experiments involving the induction of mammary tumours in rats with *N*-nitrosomethylurea (NMU) in which mispairing of $O^6$-methylguanine with thymine during DNA replication (Loechler et al., 1984) results in a single point mutation (G.C-A.T transition). A high proportion of NMU-induced mammary tumours contain Ha-*ras* oncogenes activated by G-A transitions at the 12th codon (Zarbl et al., 1985).

The organotropy of nitrosamine carcinogenesis may be partially explained by a combination of both the different activation capacities of enzyme subclasses in different tissues and the different repair capacities for $O^6$-alkylguanine and $O^4$-alkylthymine in different organs or different cell types in the same organ. Nitrosamines generally produce systemic tumours distant from the site of application, while *N*-nitrosamides produce both local as well as systemic tumours.

Activation of *ras* oncogenes (particularly at the 12th codon) has been frequently found in a range of human tumours suggesting that *N*-nitroso compounds may play a causal role in the induction of some human cancers. In human colon carcinomas and adenomas, mutations in the K-*ras* gene induced by G-A transitions at the 12th codon, the expected mutation induced by alkylating agents such as *N*-nitroso compounds is often found. Similarly, 30% of human lung cancers and 90% of acinar carcinomas of the pancreas exhibit K-*ras* mutations at the 12th codon which are mainly associated with G-T transversions (Bos, 1988).

## Nitrosamine exposure and human cancer

Experimental studies provide evidence that the biological activity of *N*-nitroso compounds in humans is not substantially different from that in experimental animals. In contrast to animal experiments, in which exposure (normally at high concentrations) to single *N*-nitroso compounds may induce cancer, human exposure results via several different sources (e.g., consumer products, foods, occupational exposure and tobacco consumption) at a wide range of different concentrations (Preussmann and Eisenbrand, 1984; Tricker et al., 1989).

Dose–response studies using experimental animals show that *N*-nitrosodiethylamine (Druckrey et al., 1963), *N*-nitrosomorpholine (Lijinsky et al., 1988) and NDMA (Peto et al., 1984), continuously administered in drinking water at exposure levels of 0.075 mg/l (0.075 ppm), 0.07 mg/l and 0.01 mg/kg body weight/day respectively, are sufficient to induce a significant incidence of tumours. In animal carcinogenicity experiments, the absence of a lower no-effect threshold (Preussmann, 1980; Peto et al., 1984) and the syncarcinogenic activity of low-concentration combinations of *N*-nitroso compounds at concentrations at

which individual *N*-nitrosamine concentrations alone would not be expected to induce carcinogenesis (Berger et al., 1987) suggest that multi-exposure to several different *N*-nitroso compounds in the diet may present a potential carcinogenic risk to humans.

In food matrices, a complex mixture of preformed *N*-nitroso compounds, their precursors (e.g., nitrosatable amines and nitrosating agents), as well as catalysts and inhibitors of nitrosation may be present. Under in vivo conditions (primarily in the gastric tract), nitrosation may also occur resulting in an increased exposure to endogenously formed *N*-nitroso compounds (reviewed by Bartsch et al., 1989). Furthermore, micronutrient deficiencies may modify nitrosamine-induced carcinogenesis with respect to both organotropism and cancer incidence. These confounding factors are partially responsible for the failure of epidemiological studies in identifying exposures to individual compounds and foodstuffs as potential risk factors for human carcinogenesis.

*Oesophageal cancer*

Experimental studies show that relatively few chemical carcinogens other than some unsymmetrical nitrosamines containing *N*-methyl substituents induced oesophageal tumours. Several nitrosamines which induce oesophageal tumours in experimental animals occur in a wide range of foods as shown in Table 1. In China, several studies have shown the potential involvement of either performed dietary nitrosamines and/or the endogenous nitrosation of dietary amines as possible risk factors for oesophageal cancer (Yang, 1980; Lu et al., 1986). Singer et al. (1986) have reported 0.1–1.3 μg/kg *N*-nitroso-*N*-(1-methylacetonyl)-3-methylbutylamine (NMAMBA) as well as low concentrations of volatile nitrosamines (mainly NDMA, NEMA, NDEA and NPYR) in foods from a high oesophageal cancer area in Linxian, China. Moldy millet and wheat flour from the same area have also been reported to contain *N*-nitroso-*N*-(1-methylacetonyl)-2-methylpropylamine (NMAMPA) (Ji et al., 1986). Molds, particularly *Fusarium moniliforme*, are common in foods from this area. Animal feeding using these mold-contaminated foodstuffs has been reported to induce epithelial hyperplasia and dysplasia of the oesophagus, and tumours of the stomach and other organs in rodents (Yang, 1980). In humans, the presence of elevated DNA $O^6$-methyldeoxyguanosine levels in oesophageal mucosa (Lu et al., 1986) suggests that exposure to environmental and/or endogenously formed nitrosamines, which on metabolism yield methyldiazonium species (NDMA, NEMA) and *N*-nitrosomethylbenzylamine), may be an important aetiological risk factor for oesophageal cancer in this area. In Kashmir, an area of high oesophageal cancer in India, the dietary exposure to *N*-nitroso compounds may also be an aetiological risk factor (Siddiqi and Preussmann, 1989).

*Gastric cancer*

The potential role of *N*-nitroso compounds (in particular *N*-nitrosamides) forms a central role in a widely accepted model for gastric carcinogenesis (Correa et al., 1975). Gastric cancer has been associated with the consumption of salted fish in Japan (Tajima and Tominaga, 1985; Howson et al., 1986) and in Japanese migrants to Hawaii (Haenszel et al., 1972). Several preformed *N*-nitroso compounds have been consistently detected in salts and preserved fish (Maki et al., 1980; Yamamoto et al., 1984; Table 1). Fish extracts, and nitrite-treated fish, from Japan induce adenocarcinomas of the glandular stomach in rats (Weisburger et al., 1980). Studies in our laboratory show that nitrosated fish produce large concentrations of direct alkylating (methylating) species. Endogenous nitrosamide formation under gastric conditions has been proposed as a potential causative agent for human gastric cancer (Mirvish, 1983). Interestingly, salted fish has also been implicated in the aetiology of gastric cancer in Norway (Bjelke, 1974) and in nasopharyngeal cancer (discussed below).

*Nasopharyngeal cancer*

Nasopharyngeal cancer is a rare malignancy in most parts of the world. However, among Chinese in Southeast China and Hong Kong it is the third most prevalent cancer in men. Nasopharyngeal cancer in this area has been associated with both Epstein–Barr virus (de-The and Ito, 1978) and the consumption of salted fish, particularly during early childhood (Yu et al., 1986). In similar studies

to the above, rats fed Cantonese salted fish developed carcinomas of the nasal and paranasal regions (Huang et al., 1978). In a more recent study, Yu et al. (1989) induced nasal cavity tumours in rats fed salted fish from Hong Kong. Several dietary surveys have shown high concentrations of volatile nitrosamines (which induce nasal and paranasal cavity tumours in experimental animals) in salted fish samples from this area (Poirier et al., 1987; Song and Hu, 1988; Table 1).

## Conclusions

There is sufficient evidence to support the hypothesis that humans are susceptible to *N*-nitroso compound-induced carcinogenesis. Thus, continuous exposure to low concentrations of several different *N*-nitroso compounds in the diet would be expected to be an aetiological risk factor for certain human cancers. Epidemiological case–control studies indicate that dietary nitrosamine exposure is an important risk factor for cancers of the oesophagus, stomach and nasopharynx.

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